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A Rapid Response System for Toxin Removal

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Introduction

Exposure to toxins and biothreat agents, and ways to mitigate the consequences is a topic of great concern. Developing safe and effective medical countermeasures that can mitigate exposure to biological or chemical threat agents for which no known antidote exists, is critical for responding to public health emergencies. Unfortunately, the list of potential agents that could be used for nefarious purposes is lengthy and, in theory, includes compounds that have not yet been identified. Developing drugs to counteract each agent is not feasible; treatments that are broadly applicable in many scenarios are necessary. This project set out to to capitalize on the body's endogenous detoxification capabilities by supplementing existing metabolic enzymes to increase the natural capacity for transforming toxic agents into less harmful constituents. By bolstering these metabolizing enzymes, which normally function to deactivate a wide range of both endogenous and exogenous substrates, we provide a means for responding to many different exposure scenarios.

The goal of this project is to develop a novel therapeutic that packages cytochrome P450 (CYP450) proteins into nanolipoprotein particles (NLPs), which when administered intravenously, will enhance the body's ability to metabolize certain chemical/biowarfare agents to inactive compounds. The CYP450 system consists of a gene superfamily of membrane-bound proteins that catalyses the biotransformation of xenobiotic chemicals and drugs, accelerating their clearance from the body [1]. Combining these enzymes with NLPs provides membrane-like support, which is critical for enzyme function [2; 3]. LLNL has unique capabilities for NLP production and extensive expertise in working with CYP450s. Success in this proof-of-principle study will lead to a new technology for rapidly treating exposures to biotoxins and other chemical warfare agents. In addition, this approach has utility for treating prescription, over-the-counter (OTC), or illegal drug overdoses, which occur thousands of times every year.

Recent studies in our laboratory have shown that membrane-bound proteins can be incorporated into NLPs resulting in stable functional proteins [4; 5]. What has not been demonstrated is the ability of the NLP/protein complex to efficiently metabolize a xenobiotic compound when administered *in vivo*. In this proof-of-principle study, NLPs were used to deliver specific CYP450 proteins to enhance the capability to detoxify certain compounds. Metabolic profiles from NLP/CYP450 treated versus untreated samples exposed to a model CYP450 substrate were quantified and compared. Successful augmentation of endogenous metabolism by the NLP/CYP450 complex will be demonstrated by a significant increase in metabolites.

Results

Protein Incorporation: Successful incorporation of CYP3A4 and the required CYP450 reductase (derived from CYP4503A4 expressing microsomes) into a purified NLP construct was achieved using telodendrimer chemistry (6). Western blot analysis showed the presence of CYP450 and the CYP450 reductase in the telodendrimer nanodisk (TND/CYP450) (Figure 1). Functionality of the CYP3A4 protein was assessed using the Promega fluorescent P450 Glo Assay (luciferin-IPA). Activity of the P450 was maintained in the in the TND/CYP450 complex for 24 days at 4°C and 12 hours at 37° C (Figure 2).

NanoDisk Characterization: Cryro-electron microscope (cryo-EM) images showed individual telodendrimer nanodisks from the TND/CYP450 complex with diameters ranging between 65 and 100 nm. Dymanic Light Scattering (DLS) data showed a fairly uniform distribution of particle size with an average diameter of particles at 130 nm (Figure 3A,B). The larger diameter seen in the DLS data compared to the cyro-EM data could be due to agglomeration of the particles.

TND/CYP3A4 functionality: To test the ability of the TND/CYP3A4 complex to metabolize a substrate, the CYP3A4 model substrate testosterone was incubated with the TND/CYP3A4 complex in vitro and the extent of metabolism was assessed by UPLC. Testosterone metabolites were separated and identified using authentic standards. The results were compared to incubations containing CYP3A4 expressing microsomes without the TND complex. The TND/CYP3A4 complex was capable of metabolizing testosterone to 6β-hydroxy-testosterone at rates comparable to the CYP3A4 microsomal positive control (Figure 4).

TND/CYP3A4 Stability: To determine if the TND/CYP3A4 complex is stable and can maintain functionality in a biological matrix, the complex was incubated with rat plasma at 37° C. Functionality and stability was assessed over time. Stability of the TND/CYP3A4 was similar to the CYP3A4 microsomal control up to 2 hours in plasma, as assessed by the P450 Glo-activity assay. Activity at 4 hr was reduced by 22% compared to the control (Table 1).

Discussion

The results show that microsomes expressing CYP3A4 can be successfully incorporated into a nanodisk complex using telodendrimer chemistry. This procedure eliminates the need for purifying the CYP3A4 before incorporation. Functionality of the complex was maintained for 24 days and 12 hours at 4° C and 37° C, respectively, indicating that these complexes are suitable for use and do not need special handling procedures to maintain activity. Their ability to maintain functionality under physiological conditions, and metabolize a substrate in vitro demonstrates their potential utility as a novel way to metabolize and detoxify toxic chemicals or drugs. Further studies are needed to determine if these TND/CYP450

complexes can maintain functionality and metabolize and detoxify compounds in vivo.

References

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Figures

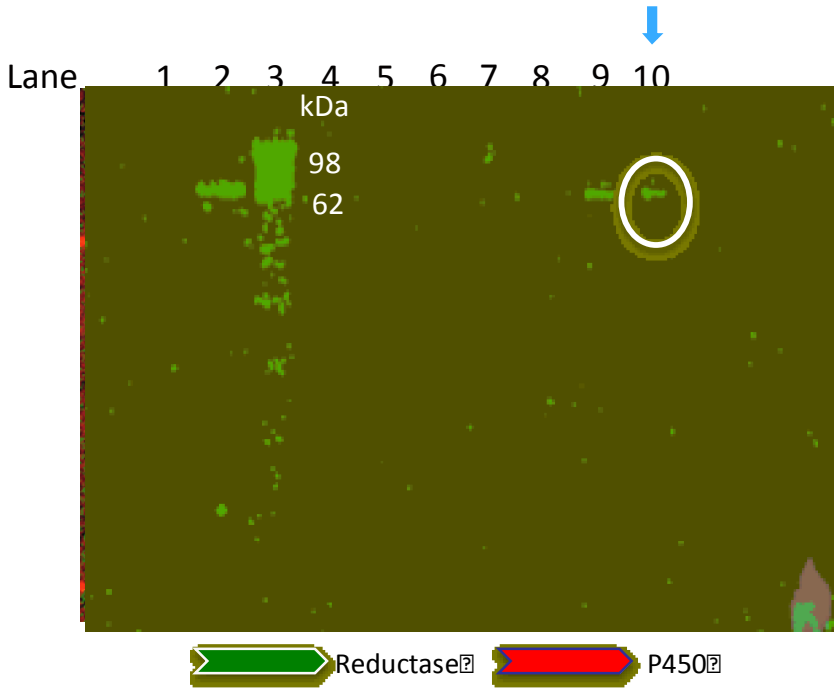


Figure 1. Western Blot showing incorporation of CYP3A4 and CYP reductase in telodendrimer nanodisk.

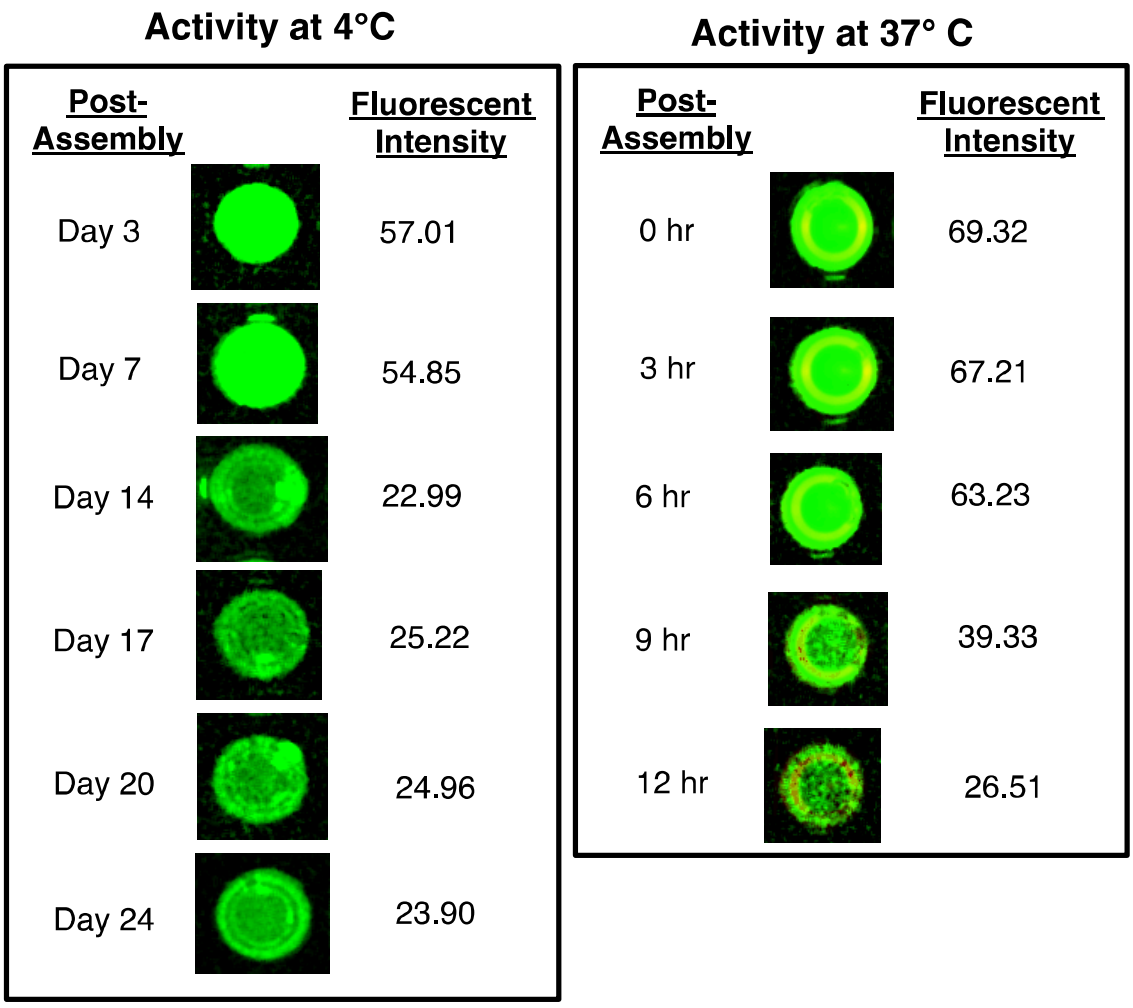
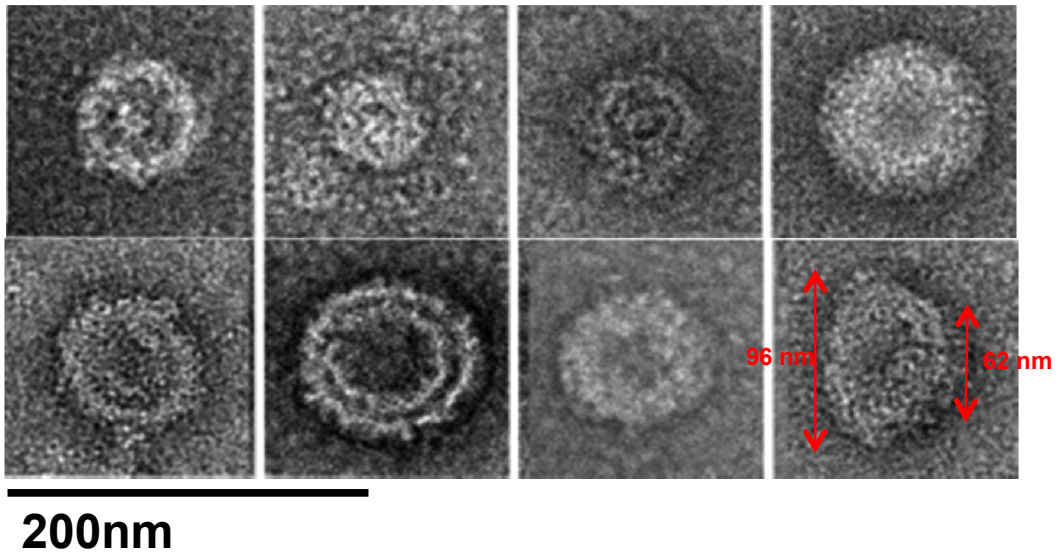
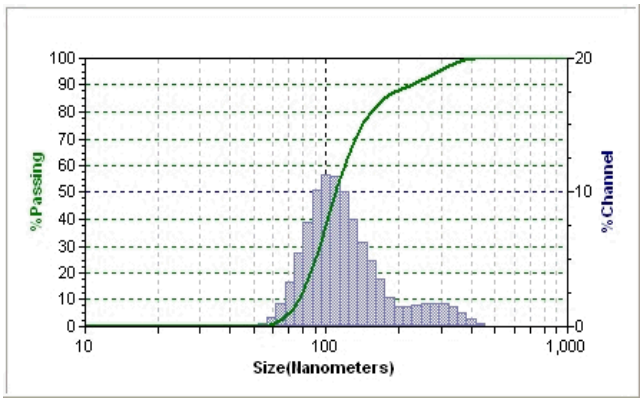


Figure 2. Activity of TND/CYP3A4 nanodisk at 4° C and 37° C over time. Activity was assessed using the Promega P450-Glo Flurescence Assay. The higher the fluorescent intensity correlates to higher CYP450 activity.

A



B



DLS Data
130 nm (average diameter)
42 nm (standard deviation)

Figure 3. Individual Cryo-electron microscope images of TND/CYP3A4 complex in potassium phosphate bufer (A), and Dynamic light scattering data (B).

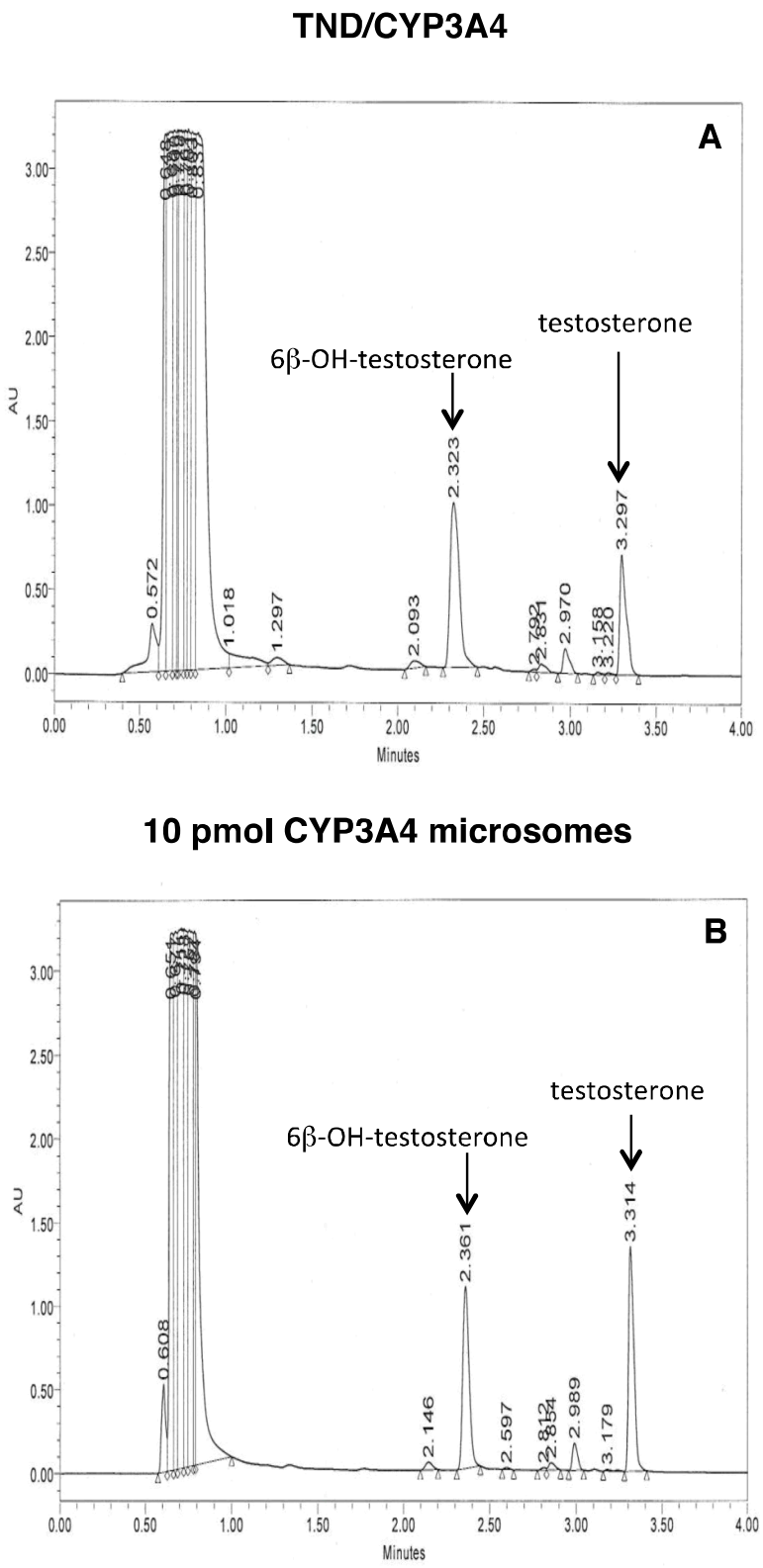


Figure 4. HPLC chromatograms showing metabolism of testosterone to 6β-OH-testosterone after incubation with (A) TND/CYP3A4, and (B) CYP3A4 expressing microsomes in vitro.

Table 1. TND/CYP3A4 activity in rat plasma at 37°C over time

	Fluorescent Intensity	
Incubation time (hr)	TND/CYP3A4	Microsomal control
0	66	69
2	67	66
4	49	63